FEVZİYE DİNDAR INVESTIGATING TEMPERATURE AND SUNLIGHT EFFECTS ON COLD PRESSED OLIVE OIL USING VOLTAMMETRIC DETERMINATION OF A-TOCOPHEROL LEVELS NEU 2021

INVESTIGATING TEMPERATURE AND SUNLIGHT EFFECTS ON COLD PRESSED OLIVE OIL USING VOLTAMMETRIC DETERMINATION OF A-TOCOPHEROL LEVELS

A THESIS SUBMITTED TO THE INSTITUTE OF GRADUATE STUDIES

OF

NEAR EAST UNIVERSITY

By

FEVZİYE DİNDAR

In Partial Fullfillment of the Reqirements for

the Degree of Master of Science

in

Food Engineering

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Approval of the Institute of Graduate Studies

Prof. Dr. K. Hüsnü Can BAŞER

We certify this thesis is satisfactory for the award of the degree of Master of Science in Food Engineering

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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ABSTRACT

Two physical factors that change the concentration of ingredients of cold pressed olive oil especially alpha-tocopherol was detected. Increasing temperature and sunlight are the factors that influenced the substance, alpha-tocopherol in cold pressed olive oil.

A-tocopherol which is found in cold pressed olive oil with high amount, was exposured to high temperature and sunlight for one hour. A-tocopherol level of cold pressed olive oil which was exposured to increasing temperature and sunlight also was measured by using voltammetric method, Differential Pulse Voltammetry. The method is quick, easy usage, cost-effective and environmental friendly. Pencil graphite electrode was used as working electrode in three electrode system.

Key words: Voltammetry; PGE; A-tocopherol; cold pressed olive oil.

ÖZET

Bu çalışmada iki fiziksel faktörün soğuk sıkım zeytin yağındaki alfa tokoferolun konsantrasyonuna etkisi ölçülmek istendi. Bu iki fiziksel faktör olan artan sıcaklık ve güneş ışığı alfa-tokoferol miktarına etki etmektedir.

Alfa tokoferolu yüksek oranda taşıyan soğuk sıkım zeytin yağı ayrı zamanlarda yüksek sıcaklığa ve 1 saatlik güneş ışığına maruz bırakıldı. Artan sıcaklığa ve güneş ışığına maruz bırakılan soğuk sıkım zeytin yağındaki alfa-tokoferol voltametri metodu olan diferansiyel puls voltametrisi ile ölçüldü.

Hızlı, kolay kullanılabilen, düşük maliyetli ve çevre dostu bir yöntemle metod geliştirildi. Üçlü elektrot sistemi içerisinde çalışma elektrodu olarak kalem elektot kullanıldı.

***Anahtar sözcükler*:** Voltametri; kalem elektrot; A-tokoferol; soğuk sıkım zeytin yağı

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LIST OF ABBREVIATIONS

ACB: Acetate Buffer Solution

AT: α-tocopherol

CV: Cyclic Voltammetry

CPO: Cold Pressed Olive Oil

DPV: Differential Pulse Voltammetry

EOO: Extra Virgin Olive Oil

HDL: High Density Lipoprotein

LDL: Low Density Lipoprotein

PBS: Phosphate Buffer Solution

PGEL: Pencil Grafite Electrode

ROO: Refined Olive Oil

ViE Vitamin E

VOO: Virgin Olive Oil

CHAPTER 1

INTRODUCTION

Olives may be generated firstly in Syria, Asia Minor, Ethiopia, Egypt, or India and dispersed to farther countries especially in Cyprus, Greece, Turkey, Syria, Lebanon, Israel, the south of Spain, France, Italy, the coast of North Africa, Latin America and Australia (Aguilera et al., 2005; Boskou, 2006). Olive oil, which is obtained from olives commonly grown in Mediterranean countries, is widely used in foods and the name of the olive fruit is *Olea europaea* in botany (Huang et al.; 2008; Ray et al, 2015).

Olive oil is indispensable for industries. The food industry, the cosmetic industry and even the healthcare industry use olive oil (Polymerou-Kamilakis, 2006). Olive oil producing countries are ranked as Europe with 2219 tonnes ,Morocco with 200 tonnes, Turkey with 183 tonnes, Tunusia with 120 tonnes, Syria with 76,5 tonnes, etc., respectively acording to statistics of International Olive Oil Council in 2018-2019 period. Oliviculture is very important in economy and tradition of Mediterranean countries (Lerma-García, 2010).

Also, olive oil has beneficial importance on human health because of its ingredients. These ingredients include saturated and unsaturated fatty acids, polyphenols, triglycerides (98-99%) chlorophylls and tocopherols (Dag et al., 2011; Lazzez et al., 2008) because of the coherence of these compounds, they give desirable nutritional property to the oil (Lazez et al., 2008).

Especially mono-unsaturated fatty acids (MUFA) are abundant in olive oils and it is associated with anti-cancerogenic function, preventing coronary problems and cardiovascular risk while reducing the level of LDL/HDL (Ray et al., 2015). Oleic acid (18:1) as one of MUFAs is found in olive oils dominantly than palmitic acid (16:0), linoleic acid (18:2) , palmitoleic acid (16:1), stearic acid (18:0) and linolenic acid (18:3) (Huang et al., 2020) and oleic acid and linoleic acid were conceived that they were accelerant healers of injured cells (Rodrigues et al., 2012). Menendez and Lupu agreed that oleic acid deactivated oncogenic action of breast cancer in 2006.

Phytosterols, α and γ-tocopherols, tocotrienols, β-carotene, flavonoids, and hydrophilic phenolic compounds (e.g., oleuropein, hydroxytyrosol, tyrosol, oleocanthal) are unsaponifiable fragment of olive oil (Ghanbari et al., 2012; Fernandes et al., 2019). It was conceived phenolic compounds have admirable potential such as oleocanthal's inflammatory preventing action (Ray et al., 2015). While the hydrophilic phenolic compounds are in olive olives, they may not found in different types of vegetable oils (Servili et al., 2009; Huang et al., 2020).

In the year 2006, Polymerou-Kamilakis claimed that olive oil has antimicrobial characteristic and preservation conditions of olive oil have significant fact. Also, olive oil has disincentive instinct to hipertension in males by decreasing systolic and diastolic blood pressure (Alvaro et al., 2006). In 2007, some researchers issued that antimicrobial activity of olive oil against foodborne pathogens and they showed that it is possible to keep the food safe when olive oil is used as a supplement because olive oil reduced level of the some pathogen microorganisms in foods (Medina et al., 2007).

Other valuable functions of olive oils are blocking of oxidation by their hydroxyl groups of phenolics e.g. hyrdroxytrosol and tyrosol (Dossi et al., 2015). The most known antioxidants of olive oils are phenolic compounds and enzymes of olive fruit (Huang, 2020). Another antioxidant α-tocopherol (AT) of vegetable oils acts as oxidative stabilizer with preventing oxidation of unsaturated bonds of fatty acids including the bonds (Sagratini & et al., 2012). Squalene, AT and β-carotene guard the other oil constituents from oxidation and they are supportive factors due to their anticancerogen functions and solutions for cardiovascular illness (Rader et al., 1997; Sagratini et al., 2013).

Recent years, the antioxidants of olive oil such as (α- and γ-tocopherols, squalene, chlorophylls, and carotenoids) were reported and they are chacterized as lipophilic antioxidants (Tuberoso et al., 2016). The antioxidant property of olive oil assited to prevent cancer risk (Menéndez et al. 2006, Diaz et al., 2019). While the olive oil incurring oxidation is unfavorable because losing of antioxidant behaviour, oxidation of the oil is measured with peroxide value and UV spectrophotometric methods (Huang, 2020).

Virgin types of olive oils have high phenolic forms that are antioxidants (Karaosmanoğlu et al., 2010) because these types are produced after mechanic process without any refining (Shendi et al., 2020).

According to Food and Agriculture Organisation (FAO) olive oils are classified as extra virgin olive oil (EOO), virgin olive oil (VOO), lampante oil, refined olive oil (ROO), olive oil composed of refined and virgin olive oils, refined olive-pomace oil and olive-pomace oil [Commission Implementing Regulation (EU), 2015].

Extra Virgin processing is significant in different types of processing for oils because of its quick processing and pressing at low temperature hence the reason it is also called cold pressed (Visioli & Galli, 2011).

EOO is higher priced than other types of olive oils in some regions of Europe (Chen et al., 2011) . After harvesting of the olive fruits, the production steps of EOO consists basically crushing, malaxation, centrifugation, storage, filtration and bottling. The major difference of EOO is in malaxation period because the malate is not reached to high temperature as refine processing as well as the maximum temperature of the extra virgin process was recorded as 42ºC (Frankel et al., 2013; Stefanoudaki et al., 2011).

Refined oil process have more critical steps than in the process of extra virgin oil and the steps are degumming 30s at 80ºC, chemical neutralization until 2% with NaOH(23,5%), physical flash neutralization at 230 ºC at 1 mbar bleaching at 90–97 ºC at 20 mbar and deodorization at 200 ºC at 2 mbar nearly 2.5 h. (Lucci et al., 2020) .

Refining process can be very critical since toxic oil syndrome happened in Spain in 1981 because of refine type of rapeseed oils (World Health Organization, 1992). Researchers from SBK Women's University analysed crude oil and claimed that refining process of this type of oil caused to decrease amount tocopherol isoforms (Ubaid et al., 2014). Following this, refining processes cause to break down the compounds in natural olives (Shendi et al., 2020), this can show virgin types of olive oils e.g EOO have high favorable contents which can defence against inflammatory, *atherogenicity*, tumor and loss of immune (Morvaridi et al., 2020; Aparicio-Soto et al., 2016) . EOO facilitates gastrointestinal problems due to its assisting to inflammation (Morvaridi et al., 2020). Researchers from SBK Women's

University analysed crude oil and claimed that refining process of this type of vegetable oil caused to decrease amount tocopherol isoforms (Ubaid et al., 2014). Hereby, cold pressed olive oil which has not any refining process, is selected for this study to detect AT and compare with refined olive oil.

Virgin olive oils are good sources for tocopherols which can be described as phenolics (Perez et al., 2019) . Vitamin E is composed of groups and tocopherols are subgroups of Vitamin E (Clark and Frandsen 1998). Vitamin E helps to preclude the heart dissease , Alzeimer and etc. because of its antioxidative function with defeating free radicals (Ubaid et al., 2014; Azeina et al, 2009). While maximum temperature is recorded as 42ºC for malaxation of fruit paste (Frankel et al., 2013), EOO is the good sources for tocopherols because refining process evokes to loss of beneficial substance in oil e.g. phenolics or tocopherols (Lucci et al., 2020).

Tocopherol is an exorbitant of oxidation by securing of unsaturated fatty acids and lipoproteins from oxidation (Sagrantini et al., 2013). α-Tocopherol(AT) is the sub-unit of Vitamin E and was searched by a lot of researchers because of its preponderant property in cells (Trela et al., 2019). AT as an example of phenols (Dossi et al., 2015) is a minor component of olive oil and an antioxidant extending shelf life of olive oils (Shendi et al., 2020).

Many methods for determining tocopherols, are gas-liquid chromatography and high performance liquid chromatography (HPLC) and gas chromatography mass spectrometry (GCMS) (Diaz et al., 2019; Bramley et al., 2000). There are some researches for analysis Turkish olive oils with NMR spectroscopic methods (Arslan& Ok, 2019). Turkish olive oils were detected for their fatty acids with Gas Chromotograpphy (GC) (Arslan& Ok, 2019) . Researchers proposed to authenticate Turkish olive oils with PCR-capillary electrophoresis and the other method for EOO is electronic nose supporting with surface acoustic wave detector and chemometrics (Arslan& Ok, 2019). Beside this FT-NIR and MIR spectroscopy were used for analysis of olive oils (Arslan& Ok, 2019). Electrochemical measurements were achieved for classification of EOO with voltammetric techniques by using screen printed electrode (Zappi et al., 2019).

However above tecniques can have disadvantages. MS and NMR have an importance to gain knowledge about structure of analyte (Silva Elipe, 2003). These techniques can involve purification problems of analyte because they are too sensitive and have complexityies (Silva Elipe, 2003). Furthermore chromotographic methods can consume long time and lower reproducibility but run times are variable recently (Dionisi et al., 1995; Pinheiro-Sant'Ana et al., 2011).

Recently, electrochemical devices gained importance in analysis methods and addition to this, the importance of electrochemical techniques is simple to use , small aparatus, easily operated and it is not expensive devices (Ruiz-Samblás et al., 2012, Dossi et al., 2007; Dossi et al., 2015; Nemiroski et al., 2014).

Electrochemical devices selectively and reversibly respond to ions or chemical compounds and generate concentration-dependent electrical signals (Karadeniz, 2008).

EOO is not exposure to high temperatures and its process only involved washing, decantation, centrifugation and filtration (International Oil Council, 2019). Cold Pressed Oil is ready to consume after mechanical processing and heat is not applied in its process (Turkish Food Codex, 2012). After production, the oil is analysed and classify according to list of the council.

In this study, it was aimed to detect alpha tocopherol (AT) in cold pressed olive oil (CPO) and determine the amount of AT component of vitamin E in CPO by electrochemical method CPO was investigated with new method. In this method a potentiostat with three electrode system was used for detection of AT in CPO.) It is aimed that AT of olive oil is detected with new method that is based on the adsoption of AT on graphite. With this developed new technique, which includes adsorption of AT on graphite rods and analysis by voltammetry, effect of temperature and effect of sunlight on AT content of olive oil was also investigated.

CHAPTER 2

GENERAL INFORMATION

2.1 Electrochemistry

The science of electrochemistry examines the transfer of electrons from one substance to another, which forms the current that will give information about the analyte being determined. Electrochemical reactions take place in electrochemical cell (Karadeniz,2008).

Quantitative determinations based on the investigation of the electrochemical behavior of the substance to be analyzed fall within the scope of electroanalytical chemistry.

In order to electrochemical reaction to occur; the solution containing the substance to be analyzed (various buffer solutions are used to provide electrical conductivity), the electrode system in which the substance undergoes chemical conversion (usually a triple electrode system) and a transducer connecting these electrodes together is necessary (Kadayıfcılar, 2007).

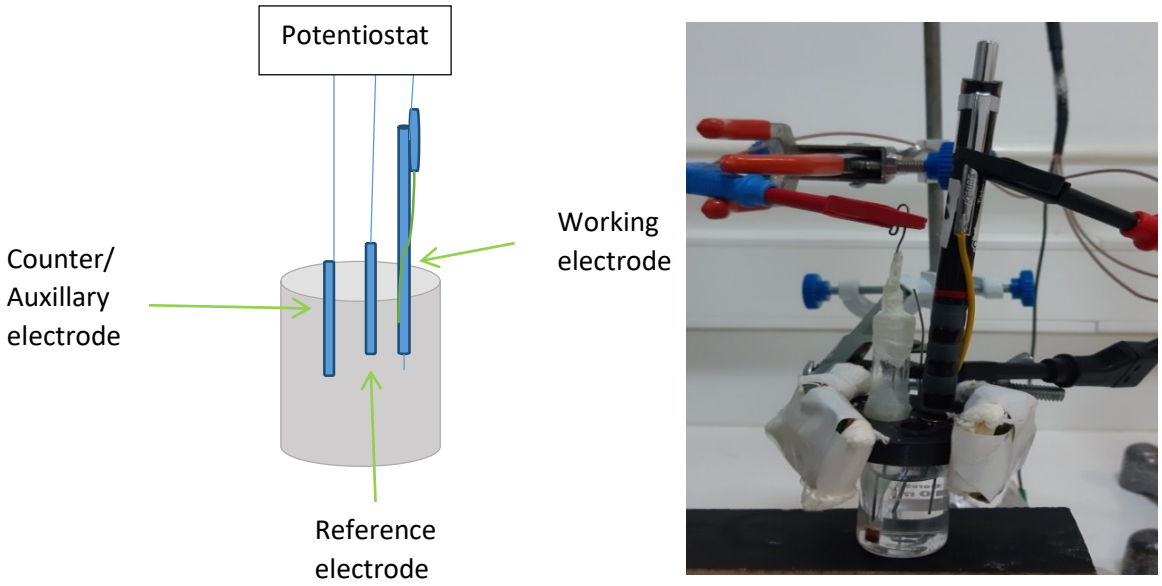


Figure 2.1: An electrochemical cell scheme and triple electrode system.

Electrochemistry involves a group of quantitative analysis methods measuring current and potential by using electrodes in an electrochemical cell (Harvey, 2000). Figure 2.1 shows an electrochemical cell with triple elecrode system.

With the electrochemical methods can be reached to very low detection limits by electroanalytical methods, a lot of information (e.g. speed and equilibrium constants of chemical reactions, adsorption, mass transfer rate and so on.) can be obtained.

Electroanalytical methods; have some advantages over other analysis methods. These methods are quick, easy, cost-effective and the other important advantage of this method is specificity of element, molecule or product that is formed after reaction to its oxidation state (Kalvoda and Parsons, 1985).

2.1.1 Electrochemical layers

When performing electrochemical measurements, the electrode is capable of delivering or receiving electrons from a type of solution layer. Thence, heterogeneous layers are formed between the electrode surface and the sample solution to be analyzed (Erdem, 2010).

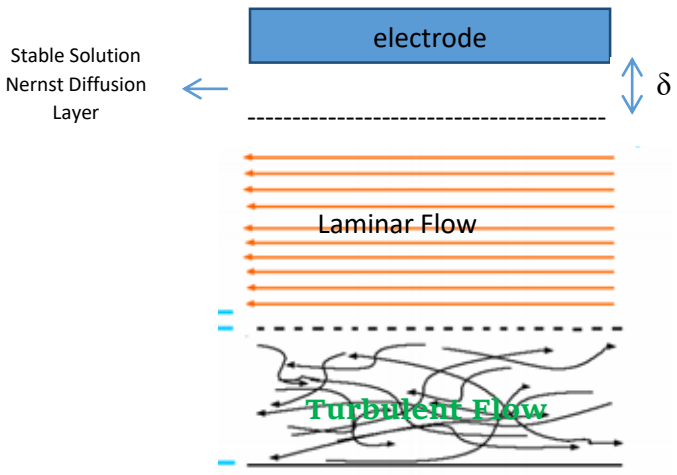


Figure 2.2: Schematic representation of the layers on the electrode surface.

Figure 2.2 shows layers on the electrode surface and these layers were explained in the following paragraphs.

a. Nernst diffusion layer: δ cm away from the electrode surface speed approaching zero due to friction between the liquid and the electrode and the result is a thin, still solution layer around the electrode. Generally, the thickness of this solution layer is about between 10^{-2} and 10^{-3} cm (Erdem, 2010).

b. Laminar flow layer: When approaching the surface, laminar flow occurs. In laminar flow, the liquid travels parallel to surface of electrode (Erdem, 2010).

c. Turbulent flow layer: It is the irregular flow layer at a distance of electrode (Erdem, 2010).

There are some electrical surveying of electrochemical layers**.** Immediately after applying a positive potential to the electrode, an instantaneous current wave will rapidly decrease to zero. (Kadayıfcılar, 2007).

Surface of the electrode were charged positively and electrical double layer was formed, densed inner layer and diffused layer (Kadayıfcılar, 2007).

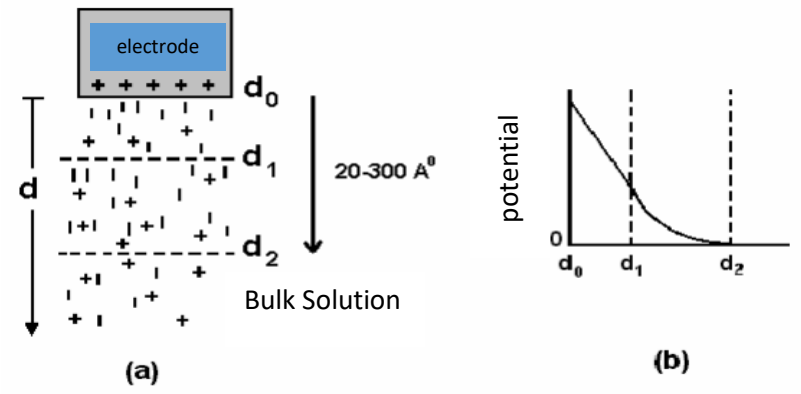


Figure 2.3: a) Electrical double layer on the suface of electrode. **b)** Exponential decreasing of potential on diffused layer.

a) dense inner layer (d0 to d1), from which the electrode surface as the distance is removed, the resulting potential decreases in direct proportion to the distance (Kadayıfcılar, 2007).

b) diffused layer (d1 to d2), the potential exponentially decreases, as the electrode surface moves away (Figure 2.3). The charges are aggregated on the electrode and formed electrical double layer (Kadayıfcılar, 2007).

2.1.2 Electrochemically mass transfer:

The substances are transferred in electrochemical cell in three ways (Skoog et al., 2014). There are three types of mass transfer ways; migration, convection and diffusion. Migration is formed because of electrical field (Skoog et al., 2014).. Convection is existed after mixing or vibrating (Skoog et al., 2014). Diffusion is a mass transfer path resulting from the concentration differences between the liquid film and the solution on the electrode surface (Skoog et al., 2014).

2.1.3 Electroanalytical methods

There are a wide variety of electroanalytical methods. Figure 2.4 shows these methods which are divided into two groups, the first is realized at the interfaces methods and the second group is realized in the whole analysis environment (Karadeniz, 2008).

Static methods (potantiometry and potantiometric titrations) and dynamic methods (controlled potential and static current) are involved in interfaces methods. Static current are sperarated into two groups, coulometric titrations and electrogravimetry whereas controlled potential are seperated into four groups, constant electrode potential coulometry, voltammetry, amperometric titrations and electrogravimetry (Karadeniz, 2008).

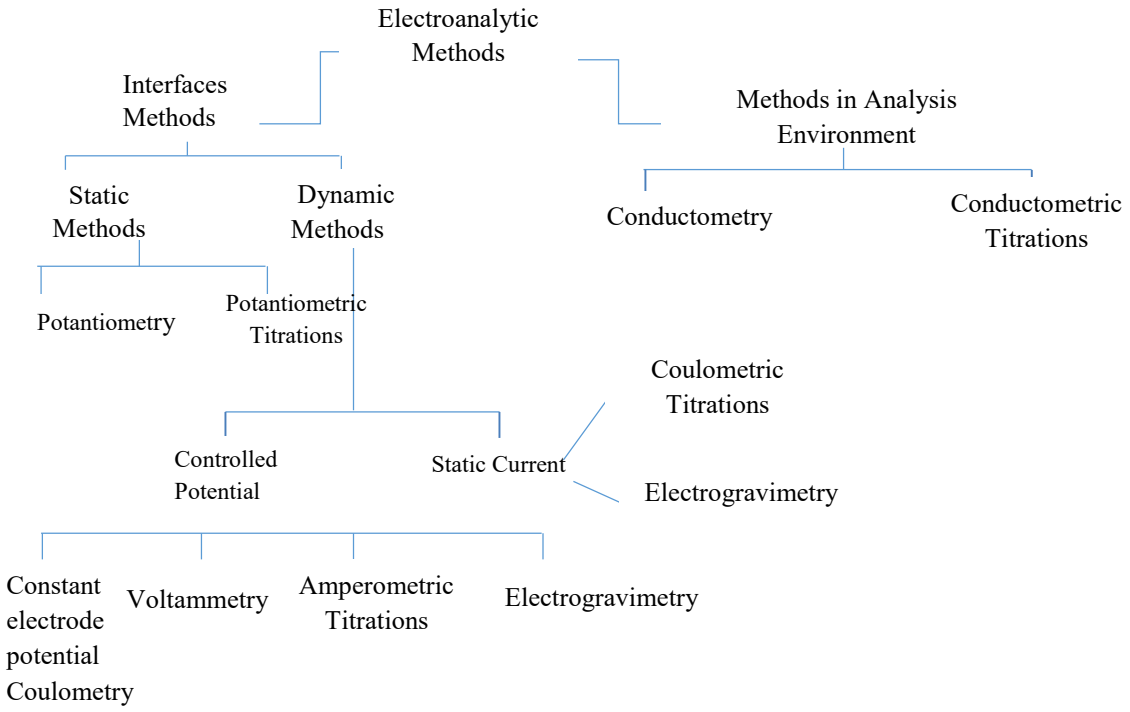


Figure 2.4: Schematic illustration of Electroanalytic Methods

Some electrochemical methods commonly used for detection of heavy metals are inclusive of voltammetry, potentiometry and conductometry (Dai et al., 2018).

2.1.4 Voltammetry and fundamentals

The electrochemical method based on measuring current as a function of the potential applied to the electrode is called voltammetry (Skoog et al., 1996). Curves drawn between the current measured against the applied voltage are called voltammograms (Wang, 1994).

In voltammetry, the limits of the potential range that can be applied to the electrode to examine the electrochemical behavior of any material depend on the working electrode used and the types of solvent and electrolyte used.

In the early 1920s, voltammetry is developed by the Czech chemist Jaroslav Heyrovsky and it is named as Polarography, a special type of voltammetry (Kadayıfcılar, 2007).

Oxidation or reduction of matters, adsorption of surface and transition of electrones are investigated in voltammetry (Wang, 1994; Kadayıfcılar, 2007).

1)Voltammetric Signals

The shape of the four most commonly used excitation signals in voltammetry is given in the Figure 2.5. These are linear scanning, differential pulse, square wave and triangular wave (Skoog, 2013).

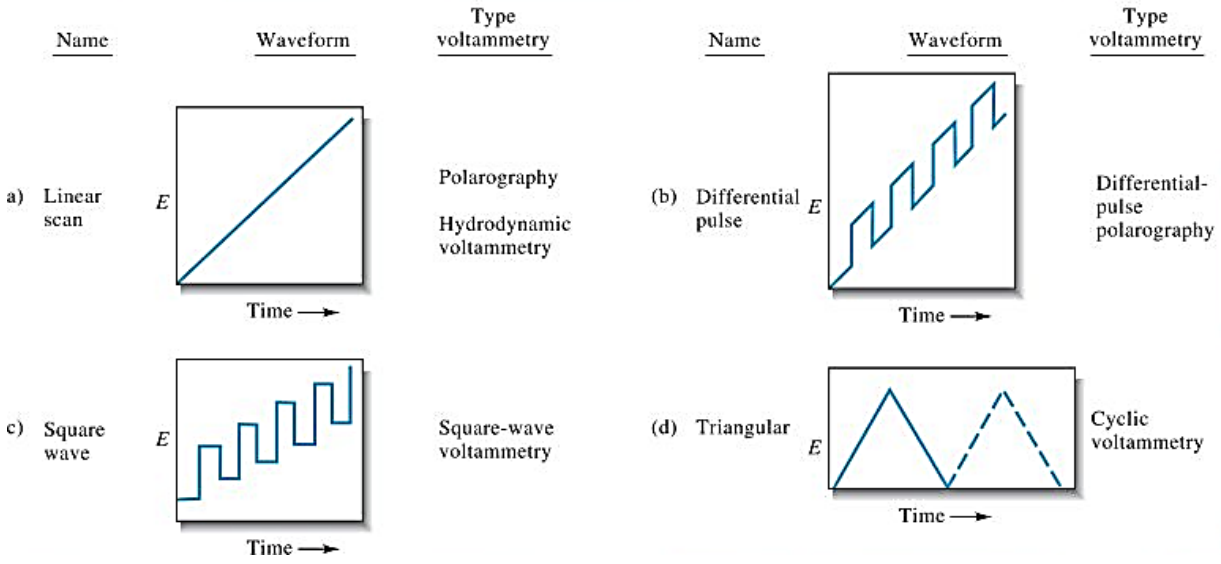


Figure 2.5: Common types of Voltammetric signals (Skoog, 2013).

2)Voltammetric Instruments

Figure 2.6 shows electrical structure of voltammetric instrument (Arıksoysal, 2006).

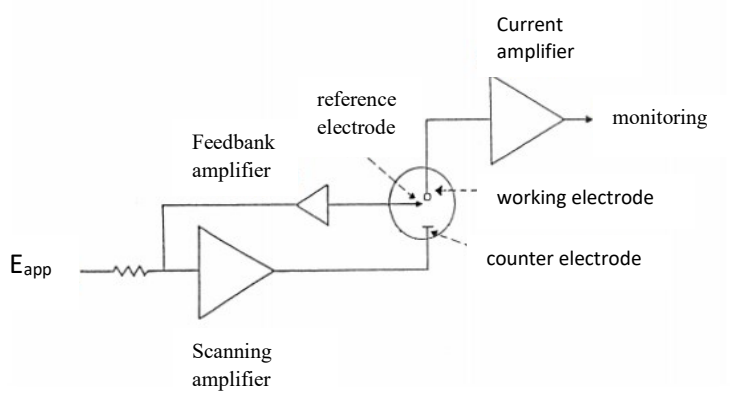


Figure 2.6: Schematic view of triple electrode system of potentiostat

The functions of the system can be explained briefly with this paragraph. The current amplifier connected to working electrode and potentiostate keeps the working electrode at a certain potential. Biosensor design may vary according to the purpose of the study. The reference electrode is connected to potential control circuit whose potential remains constant throughout the experiment and it precludes the currents since the control unit of reference electrode has high resistance (Arıksoysal, 2006). The amplifiers function as counting and providing of currents between refence electrode and working electrode and retaining the potential difference at a certain level. And the third electrode is auxiliary/counter electrode (Karadeniz, 2008) allows the transfer of electricity through the solution to the working electrode and it has not effect on magnitude of potential (Arıksoysal, 2006; Karadeniz, 2008).

3)Electrodes In Voltammetry

For general definition, electrodes are electronic conductors and they tend to ionic conductors (electrolyte) in electroanalytic system via transporting electrons (Bard& Faulkner, 2001).

Three types of electrodes are used in electroanalytic system and these are shown below in the Figure 2.7.

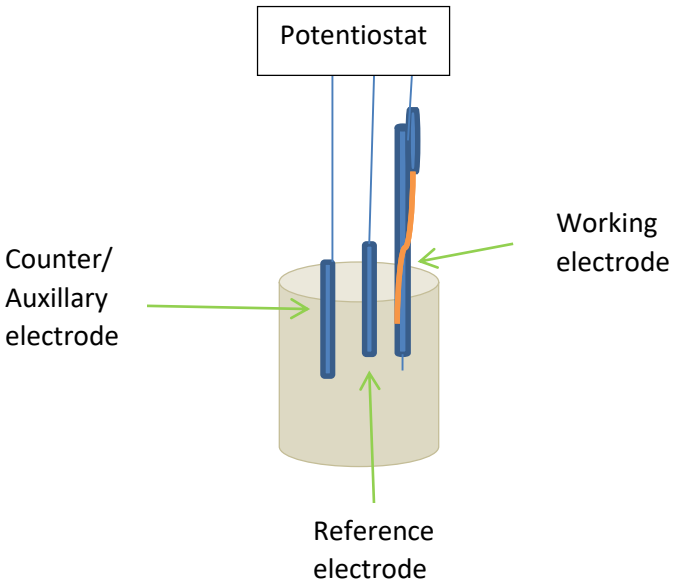


Figure 2.7: Three types of electrodes

***1) Working Electrodes*:**

The conductive material used in the construction of the working electrode is an inert metal such as platinum or gold; carbon paste, nickel voltammetry electrode, carbon microelectrode, gold microelectrode, platinum microelectrode, palladıum voltammetry electrode, pyrolytic graphite or glassy carbon voltammetry electrode, silver voltammetry electrode (Bioanalytical Systems, 2001); such as tin oxide or indium oxide may be a metal or semiconductor coated with a mercury film (Karadeniz, 2008). Pencil graphite electrode is used for this research.

The types of working electrodes were shown below.

a.Pencil Graphite Electrode:

Pencil graphite electrode was used in this research. Pencil graphite electrodes are inexpensive and have electrical conductivity. Processing of a pencil is composed of graphite and clay in water and is treated to heat up to 1000ºC to achieve rigidity. After that procedure,

it is subjected to wax bath to attain regular surface (Ishida et al., 1997; Torrinha et al., 2018). Components of a pencil graphite are graphite (68%) , clay (26%) and wax (5%) with approximate percentages.

b. Carbon Paste Electrode:

The carbon molecules in the graphite powder are arranged in planar and aromatic rings. These layers are connected by weak ∏ bonds and there can be a rapid exchange of electrons between them. CPE is widely used because of its easy surface renewal, cheapness and low residual currents (Erdem, 2010).

c. Glassy Carbon Electrode:

Glassy carbon is obtained by carbonization of phenol / formaldehyde polymers or polyacrylonitrile temperature between 1000- 3000 ° C. Although GCE is not commercially suitable for electrode production, it is frequently used because it has very good mechanical and electrical properties and has a wide potential range, does not react with chemicals and generally provides repeatable surfaces (Erdem, 2010).

d. Screen Printed Electrode:

These electrodes were widely used in DNA studies as biosensor (Lucarelli vd., 2002, Marazza vd.,1999, Erdem, 2010).

e. Metal Electrode:

Platinum and gold (Carpini et al. 2004; Herne and Tarlov 1997; Özkan et al., 2002) are the most preferred electrodes.

***2) Reference Electrode*:**

It has stable potential during analysis because it is distanced from analysed sample. The types of reference electrodes are silver/silver chloride (Ag/AgCl) electrode, saturated calomel electrode and Hg-Hg(1) Sulfate electrode,… (Krajewska et al, 2008; Foertsch et al., 2011; Li et al., 2006; Michalkiewicz et al., 2002).

Properties of reference electrodes are easy to prepare, the coefficient of variation of the potential with temperature is small, reversible characteristic within a certain current range, small currents through it even when the voltage stays constant, being an unpolarable electrode, potential does not change over time and quickly read an accurate and repeatable potential values (Erdem, 2007).

a.Ag/AgCl Electrode:

It is commonly used reference electrode. A silver (Ag) wire coated with Silver Chloride (AgCl) with electrolysis or holding in concentrated Hydrochloride (HCl) solution for a while and the coated wire is immersed in a chloride containing solution (Karadeniz, 2008).

***3) Auxiliary/Counter Electrode**:*

It is the opposite electrode, usually a helical platinum wire or a pool of mercury, which allows the transfer of electricity to working electrode. (Erdem, 2010).

4)Voltammetric currents (Faradaic And Non Faradaic Currents)

The current is transferred from the electrode solution interface by two types of processes. The first one is the oxidation reaction in one of the electrodes, and the other is the reduction reaction. The current is carried during oxidation and reduction process and it is called faradaic processes because they comply with Faraday's laws.

A charging current comprising a charged double layer at the electrode / solution interface. By immersing an electrode in a charging solution and charging with a negative charge,

positively charged ions in the solution are drawn towards the electrode. This creates a potential difference at the interface. An electrical double layer is formed in this region by the accumulation of reversed loads on both sides of the interface. The resulting double layer acts as a capacitor. A current is generated to charge this capacitor, even if there is no substance to be oxidized or reduced. This current is not connected to the reaction and originates from the system, which is called a non-faradaic capacitive current ic (Skoog et al., 1996& Wang 1994).

5)Voltammetric Techniques

The types of voltammetric techniques are shown.

a. Cyclic voltammetry

The direction of the initial screening can be either negative or positive depending on the sample composition. Generally, the cycle time is in the range of 1 ms or less to 100 s or more (Skoog et al., 2014).

With detailed examination of cyclic voltammograms, it is possible to understand at what voltages and how many steps a system is reduced and oxidized, whether it is electrochemically reversible, whether the electrode reaction goes with a chemical reaction, whether the reduction or oxidation products are stable, whether the substances involved in the electrode reaction are attached to the surface (Erdem, 2010).

b. Differential pulse voltammetry and polarography

In normal pulse voltammetry, the current detected at the end of the pulse contains a small amount of capacitive components. In order to further reduce the share of this component in the measured current and raise the selectivity, it was attempted to measure the currents at the beginning and end of the pulse and take their differences. The method working with this technique is called differential pulse voltammetry (DPV) (Tural et al. 2006).

Sensitivity of DPV is high in voltammetric techniques (Skoog et al., 2014).

2.1.5 Sensor

The sensor is a small detector used for direct measurement of analyte in a sample matrix. Such a device should be reversible and capable of continuous response, without affecting the sample under examination. A sensor consists of three basic components.

 Recognizing section,

 The transducer section that converts the interaction between the recognized and the recognized into a measurable signal,

 Electronic section,

The sensors that convert the interaction between the recognized and the recognized into electrochemical measurable signals are electrochemical sensors (Erdem, 2010).

2.2 Vitamin E

Vitamin E (ViE) was approved by scientists in 1922 (Lu et al., 2015) . Since it can not formed naturally in body, humans need to take daily (Ruperez et al., 2001). It has eight members of tocochromanols (Hussain et al., 2012; Barros et al., 2020). ViE is a huge molecule, has eight isomeric forms and its structure is dominated with methyl groups as lipophilic parts (Min, 2007) altough it has hydrophylic strucrure on chromanol ring because of hydroxyl group on it (Quinn, 2007). It can be found in vegetable oils e.g. palm oil, olive oil, also in animal sources such as fishes, vegetables such as spinach, soybean, varieties of grain and seeds, rice bran etc. (Ruperez et al., 2001).

ViE is found in adipose tissues, liver, muscles and fat cells in the body and α- tocopherol was present with the highest concencentration in the ViE isomers in tissues (Quinn, 2007).

1.Physical Properties of Vitamin E

The melting point is 2.5–3.5 ºC and the boiling point is 200ºC approximately. The ultraviolet absorption spectra of the tocol and tocotrienol in ethanol 292–298 nm (Quinn, 2007) and maximum ultraviolet light absorbance (λ_{max}) is at about 220 nm (NE Craft, 2016). ViE is fluorescent with an emission maximum about 325 nm in hydrophobic environments (Quinn, 2007). The spectral shift associated with changes in environment is a useful method for investigating the interaction of ViE with other molecules (Quinn, 2007).

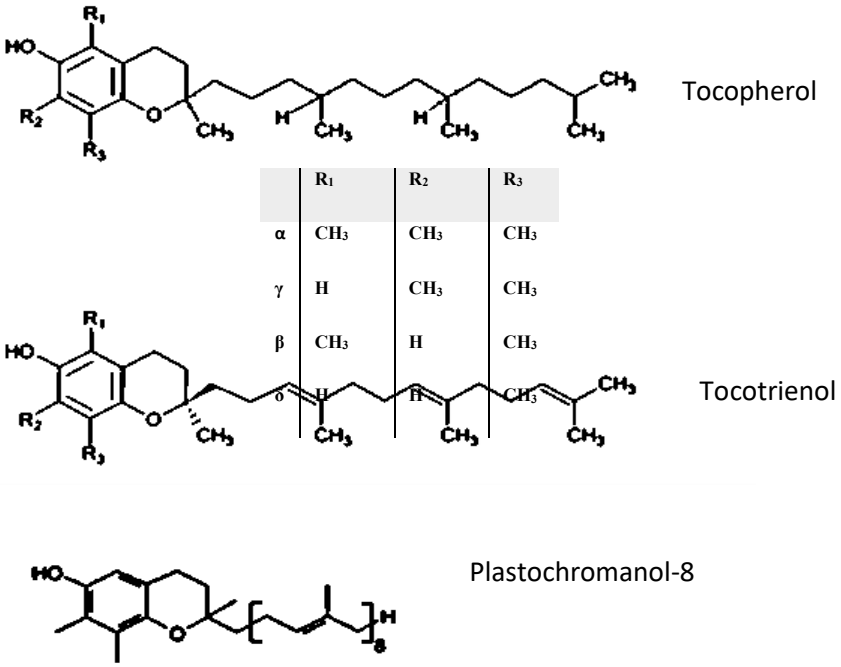
2.Structural Properties of Vitamin E

Since ViE has hydroxl group on chromanol structure, this support the big molecule to gain polarity (Quinn, 2007).. Also ViE has phytyl group as a side chain which gives to the molecule non-polarity property (Quinn, 2007). Altough it has the polarity property, the non-polarity character is dominated on the whole molecule (Quinn, 2007).

3.Isomers of Vitamin E

ViE is the proverbial fat-soluble vitamin in vegan diet oils (Ruprez 2001, Mikheeva and Anisimova 2007) and it subsumes eight isomers α-, β-, γ-, and δ tocopherols and α-, β-, γ-, and δ tocotrienols (Karl and Josef Dietz, 2003). General structure of ViE can be determined with two structures, chromanol and phytyl (Karl and Josef Dietz, 2003; Quinn, 2007). While ViE has eight isomers, four types of tocopherols and four types of tocotrienols, the isomers are dissociated from their linkages to phytyl chain of ViE (Sen at al., 2000; Quinn, 2007). Additionally to the eight isomers, ViE has one more subgroup plastochromanol-8 which is appeared from derivation of γ-tocotrienol (Trela& Szymańska, 2019) . Tocotrienols spreaded less than tocopherols in natural products and the third group of isomers, plastochromanol-8 is found in the leaves more than seeds (Trela& Szymańska, 2019). A-Tocopherols (AT) are dominated in other groups of isomers, thus there are a lot of researching about AT in scientific areas (Trela & Szymańska, 2019). Plants photosynthesize and form tocochromanols whose amount varies among species (Falk & Munné-Bosch, 2010;

Trela & Szymańska, 2019). Tocochromanols play a part as an oxidant in lipids (Trela & Szymańska, 2019).



	R_1	R_2	R_3
α	CH_3	CH_3	CH_3
γ	H	CH_3	CH_3
β	CH_3	H	CH_3
δ	H	H	CH_3

Figure 2.8: Structure of tocopherols (α, β, γ and δ-), tocotrienols (α, β, γ and δ-) and plastochromanol-8.

4.Importance of tocopherols and AT

Daily intake of tocopherols 8 to 10 mg for a person. According to FDA (Food and Drug Administration), the sub-unit of ViE, tocopherol is in generally recognized as safe (GRAS) list (FDA, 2020). Tocopherols was defined as antioxidants with a code, E306 in UK (FSA, 2020).

According to current dietary recommendations, the recommended dietary allowance (RDA) of ViE (α-tocopherol) is 15 mg, and the estimated average requirement (EAR) is 12 mg (NIH, 2020).

Tocopherols are scavengers while they are exorbitant of reaction of unsaturated fatty acids to turn to peroxyl radicals which can evoke to uncontrolled reactions (Engelking, 2015).

AT amount is the highest content in the isomers of ViE. There are a lot of searching about AT as dominant isomer of ViE (Trela & Szymańska, 2019).

Seperation of AT from its source is easier than the β and γ types of tocopherols (Malloy and Kane., 2015).

5.Vitamin E (alpha-tocopherol) content according to olive oils' types

Tocopherols' content found in ROO, VOO, EOO and COO are 96, 100-300, 185-212, 142-344 with the minimum and maximum contents and unit mg/kg respectively showing on Table 2.2. (Lucci et al., 2020; Psomiadou et al., 2000; Kamal-Eldin et al., 1997; Gimeno et al., 2002).

Table 2.2: Avarage Tocopherol content according to four types of olive oil .

Types of olive oils	Interval of contents of tocopherols(ATs)	
Refined Olive Oils	96 mg/kg	
Virgin Olive Oils	100-300 mg/kg	
Extra Virgin Olive Oils	185-212 mg/kg	
Crude olive oil	142 - 344 mg/kg tocopherols	(Predominant forms of tocopherols are ATs)

2.2.1 Beneficial properties of vitamin E

When ViE is thought as antioxidant there are variable effects with restaining uncontrollable radical chain reactions that appeared from lipoproteins and fatty acids. Theoritical explanation about this fuction of ViE are explained with structure of the ViE isomers has common chromanol ring which has a hyroxyl group at least and the hydrogen catches the unpaired electrones of radicals, so ViE limits the reaction of the radicals with another organic molecules (al-Attar, 2013). Reactions of ViE as an antioxidant are attributed to scavege of free radicals. The radicals have strong activation to react other molecules because of characteristics of electrones but they evoke undesirable reactions (Devasagayam et al., 2004). Unnatural events like pollution, X-Ray as well as overloading of some minerals in the body can cause formation of the radicals (Devasagayam et al., 2004).

ViE avoids lipids to oxidize in plant oils therefore it supplies stable conditions for the oils (Borges et al., 2017). ViE (AT) limits lipid peroxidation when mice were fed with AT.

ViE have a lot of good effects for health and inhibiting of enzymes are one of the good effects because they are enzyme inhibitors that are involved in some cell reactions of cerebrum, immune system, etc (Malloy&Kane, 2015). ViE level of blood is effectual for treatment of discomfort changes in the body such as high glucose in the blood, high blood pressure and cardiovascular problems (Wong et al., 2017; Ricciarelli et al., 2000).

Tocotrienols which are part of ViE have represented prominent ViE activity as a protector of a brain damage dissease, carotid atherosclerosis (Tomeo et al., 1995; Sen et al., 2000). While they supply good support to cholesterol balance in cells, they have anticarcinogenic properties also (Khanna et al., 2010 ; Trela & Szymańska, 2019).

Also, α-Tocopherol(AT) is marked as an antioxidant compound for years and scientitists searched about antioxidant behaviour of AT (Boscoboinik et al., 1994; Azzi et al., 1995; Sen et al, 2000). About 30 years before, AT was reported that it was used as an antioxidant with the other compounds, probucol and lipoic acid and prevented the cells against cytotoxicity and cell injury (Han et al., 1990; Murphy et al., 1997; Sen et al., 2000; Sano et al., 1997). On the other hand, AT is found that it heals memorial problems on mice (Kandlur et al., 2020; Nagai et al., 2002).

However AT has a fascinating property with docking action of toxic enzyme because it can bind to toxic compound after the secretion of venomous animal e.g snake which has phospholipase A2 (PLA2) causing a lot of problems (neurological, etc.) in the body (RS & Gurunathan, 2020) .

Tocochromanols which are parts of ViE groups treat as oxidant to prevent oxidation of lipids and protective to neuralogic system (Trela et al., 2019). ViE with B group vitamins such as B_6 and B_{12} and Iron supply advantages to memory of human brain and ViE acts as an antioxidant for Vitamin A (Whitney and Sharon, 2009; Al-attar, 2013).

Usage of ViE can be unnatural while it can added in foods and produced synthetically. 8 Stereoisomers (*RRR*-, *RRS*-, *RSR*-, *RSS*-, *SRR*-, *SRS*-, *SSR*-, and *SSS*-) of AT are synthetical forms while RRR- AT is produced naturally (Zhao et al, 2015). If the synthetic or natural forms of AT are consumed , γ- tocopherol level of human plasma can be influenced (Zhao et al, 2015). The natural tocopherols are activated easily according to synthetical succinated and acetated forms of AT which are supplemented in foods (Al-attar, 2013) .

Another group of ViE is plastochromanol-8 and the scientists claimed that they have strong antioxidant capacities (Trela & Szymańska, 2019).

2.2.2 Other methods using for vitamin E analysis or extra virgin olive oil analysis with basic principles

The members from Federal University of Vicosa investigated isomeric structure of ViE by HPLC with fluorescence detection (Pinheiro-Sant'Ana et al., 2011). Researchers from China did a study on Determination of ViE Isomers in Vegetable Oil by HPLC with Fluorescence Detection (Yang et al., 2018).

Researchers from German Institute for Human Nutrition investigated ViE substituents by using HPLC- ECD (High Pressured Liquid Chromatography - Electrochemical Detection) method (Lodge et al., 2000).

a.High Pressure Liquid Chromotography (HPLC): High Performance Liquid Chromatography (HPLC) is based on the principle that the components dissolved in a liquid enter the variable external support support in a column, and different variable components leave the column as a result of moving at different speeds. Normal phase and Reverse phase are the types of methods for chromotography (McNair et al., 2019)..

b.Gas Chromotography (GC): Chromatography is a method of separating the components based on the principle that two or more components in a mixture move at different speeds between different phases due to their different interactions between these phases through a stationary (stagnant-adsorbent) phase with the help of a mobile (carrier-mobile) phase (McNair et al., 2019).

c.Liquid Chromotography (LC): Liquid Chromotography – Mass Spectrometry (LC-MS): This technique is including HPLC principles, compounds of samples are dissected by ionizing and dissociation with the ions according to mass/ charge ratio. Selected ions is diverted to a tube detector which classifies ions (McNair et al., 2019).

2.3 Oxidation Reactions of AT

Primarily, water lose an electrone and hyroxyl radicals presenting on working electrode suface is caused to oxidation in electrochemical process (Erdem et al., 2019).

Free radicals with unpaired electrones evoke molecules to involve in reactions easily. Because of alpha tocopherols' radical scavenger function in cold pressed oil, α-tocopherols shows its funtion and can break lipid oxidation chain. After turning ATyl (alpha - tocopheroxyl radical), ATs derivatives are formed e.g. 6-O-lipid-α-tocopherol, α-tocopherol spirodimer, α-tocopherones, AT trimers and α-tocopherylquinones showing in the Figure 2.9 (Yamauchi, 1997). Figure 2.9 shows structure of ATs and derivatives of AT after reacting with lipid radicals.

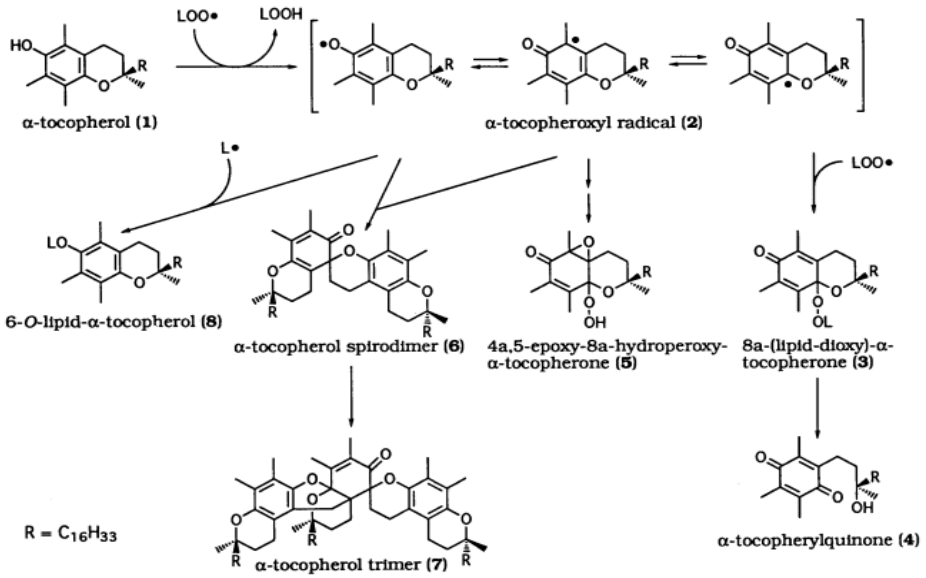


Figure 2.9: Derivatives of A-Tocopherol in autooxidation of unsaturated lipids. LOO* lipid peroxyl radical, L* carbon centered lipid radical, LOOH lipid hiydroperoxide (Yamauchi, 1997).

CHAPTER 3

RELATED RESEARCHES

While the aim of the study is the determination of AT (a sub group of Vitamin E) level in extra virgin olive oil(EOO) with electrochemical technique, the literature was searched and summarized in this chapter.

Researhers from Romania searched EOO which was detected with voltammetric method, square wave voltammetry and it was aimed if any adulteration was in EOO. Different types of oils of vegetables, sunflower, soybean and corn was compared with different concentrations in EOO by the voltammetric method. There was an distinguish instrument which is carbon paste electrode as working electrode in three electrode tecnique. The study could proved dissimilarities in the mixed oils via their methods (Apetrei &Apetrei, 2014).

Diaz and her team were investigated the origin of extra virgin olive oils and compare Spanish and Portuguese extra virgin olive oils, polar compounds gain importance and they arise in electronic tongue technique. This technique is combined with potentiometry and Ag/AgCl used for reference electrode. Furthermore, statistical analysis is supported via MANOVA (multivariate analysis of variance) and ANOVA (analysis of variance) (Diaz et al., 2014).

Michalkiewicz team investigated tocopherol isomers (α, β, λ types) and α- tocopheryl acetate in acetic acid solution by voltammetric method. Differential pulse and square-wave are used as sensitive voltammetric applications. The researchers claimed that fatty acids could be in medium without interaction. Polycrystalline platinum (1.5 mm diameter) as working microelectrode and acetic acid solutions containing 0.1 M CH3COOK and 0.1 M NaClO4 as the background electrolyte are used. They could obtain oxidation conditions of the types of tocopherols (Michalkiewicz et al.,).

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Researchers from Spain did analysis for ninety Greek virgin oils and they found that the oils containing with minimun 98 and maximum 370 mg/kg α-tocopherol amount. The amount of α-tocopherol had not efficiency on their extraction condition. As well as, they brought an idea about good oil handling importance for α-tocopherol amount (Psomiadou et al., 2000).

Researchers aimed that Vit E isomers, α, β, δ, γ- tocopherols can be seperated by voltammetric method. The method included three electrode system containing glassy carbon as working electrode and they compared direct current with differential pulse voltammetry (DPV). Thet could succed to prove DPV can give more reliable results. Beside this, they were willing to know that substances effecting on experimental condition (Diaz et al., 2004).

Three groups of antioxidants including tocopherols, tocotrienols and plastochromanol-8 in ViE were investigated in this study. They could detect tocopherol content in plant seed oils and AT is at high level. They used HPLC to detect isomers of ViE including tocopherol in vegetal oils and UV–VIS spectrophotometer to measure absorbance of oils in DPPH (2,2-diphenyl-1-picrylhydrazyl, 0.5 mM) for antioxidant behaviour of oils. They could give levels of AT with the unit mg/100 g oil (Trela & Szymańska, 2019).

The study has an aim for explanation of Arbequina oils which can have unsimilarities, if they are produced some parts of Brazil or Spain. Nine cities from Spain and two states from Brazil where the olives are producted were selected to collect ExVO. They analyze three types of compounds and one of them is AT. They were aware of different contents of the three types in oils from different regions. The analyzed compounds are CoQ_{10}, Phenolics and AT. They reported the amount of AT in EOO according to some areas of Brazil and Spain and the environmental conditions can be effective on AT level in ExVO during the growth of fruits. Their methods are HPLC with electrochemical detector and UPLC system. ANOVA was used for statistics also (Borges et al., 2017).

In 2013, the researchers investigated a voltammetric technique that they defined as electronic tongue to show diversities of different types of oils. They illuminated that the contents of oils can be analysed by their voltammetric method and they can show diversity of oil. They can qualify the oils and they can find the oils which can be mixed with other types of oils. The voltammetric application with three electrode (gold, platinum and saturated calomel) is cyclic voltammetry. Five types of plant oils are used in sampling. Three analysis methods are used to classify them (Men et al., 2013).

The scientists detect oils with pencil-paper based electrochemical system. After application of cyclic voltammetry to oils, they unveiled that AT content of oils can be evaluated. The oils can be seperated from each other, if they belongs to different plants or plant seeds. EOOs are analyzed and results are reproducible with 13% (Dossi et al., 2015).

Ok distinguished the comestible oils brought from some countries (Italy, Brazil, Argentina, etc.) . Two compounds including tocopherol in oils support to distinguish the oils that are analyzed by H- NMR technology with statistics program ANOVA (Ok, 2016).

A thesis about α-tocopherol which is exposured to hot temperatures shows that AT can be damaged by heat for certain time interval. AT level in plant based oils is determined by HPLC and DU800 UV/visible spectrophotometer (DPPH) and results were evaluated statistically. The researcher compared two heat applying methods to these oils which one of them is classified as EOO type. The two applied procedures are cooking with pan and cooking in oven (Hasan Al-attar, 2013).

CHAPTER 4

MATERIALS AND METHODS

4.1 Equipments

All devices and equipment used during measurements are ph-meter, magnetic stirrer, vortex, potentiostat (Metrohm Autolab B.V. Kanaalweg 29/G 3526 KM, Utrecht), working electrode (pencil graphite electrode), auxiliary electrode (platinum electrode), reference electrode (Ag / AgCl).

4.2 Materials and Samples

Acetic Acid (99-100%), Sodium hydroxide, Dipotassium monohydrogen phosphate, Potassium chloride and Potassium dihydrogen phosphate are obtained from Merck, Sodium chloride are provided from Sigma, Olive oil (Cold Pressed) are obtained from local producers, and Vitamin E Supplement (includes alpha-tocopherol standard) are supplied from pharmacy. Addition to this, Tombow Lead (0.5 mm with a radius) were used in this analysis as working electrode material.

Cold pressed olive oil and refined olive oil collecting from Güzelyurt in Cyprus as local production were used for analysis.

4.3 Preparation of Materials and Samples

4.3.1 Oil samples

Cold pressed olive oil was collected from Güzelyurt in Cyprus as local product was used for analysis. Olives were collected during October and November which brought to an olive oil fabricate. Then collected olives served with cold pressed process that kept the temperature of the product colder than refined process. Oil samples were hold in glass bottles at room conditions.

Oil was filled in Ependorf tubes that had volumes of 200µl. Activated graphites were placed in tubes of oil one by one and held in them for 30 min, then they are being taken out for drying for 30 min. After all these preliminary steps, measurements were started.

4.3.2 Tocopherol samples

As a Vitamin E supplement which contains d- α- tocopherol (200 IU) was used. It is produced by Koçak Firm in Turkey. The brand of the pharmaceutical product was Evicap.

Exipients of the Evicap are soy oil, methylparaben sodium 0.437 mg, propylparaben sodium 0.109 mg, gelatine, glycerine and sunset yellow.

Evicap is filled in Ependorf tubes that have a volume of 200µl. Activated graphites are placed in tubes of Evicaps one by one and held in them for 30 min, then they are being taken out for drying for 30 min. After these steps, measurements are done.

4.3.3 Buffer solutions

Deionized water was used for buffer solutions. The buffer solutions were strored in refrigerator at 5-8 C.

a. Acetate buffer solution

14.45 ml acetic acid with 99% v/v and 0.84 g NaCl were added in deoinized water in a bottle about 250 ml and solution was arranged to pH 4.8 by addition of 0.1 M NaOH. Finally it was completed to 500 ml with the water. Acetate buffer with the molarity concentration of 0.50 M was hold in a glassy bottle in a refrigerator.

b. Phosphate buffer solution

0.05 M Phosphate buffer solution was used. 0.68 g of KH_2PO_4, 3.48 g K2HPO4 and 0.84 g NaCl were added to deionized water and completed to 500ml with deionized water. The pH of solution was arranged to 7.4.

4.4.4 Aparatus and electrodes

Pencil leads' brand is Tombow which is produced in Japan and pen was supplied from Rotring Firm. Pencil graphites were cut in the middle of leads which have the length of 6 cm and it was placed in the pencil and outer part of the lead of the pencil is 1.5 cm in buffer solution. The brand of pen which is used as working electrode is Rotring which has full conductive characteristics of metal parts for electricity. Platinum electrode was chosen for auxillary or counter electrode. Platinum electrode is held in a plastic pot and it is cleaned with deionized water before it will used. Ag/AgCl in 3M KCl was chosen for reference electrode. The electrode was standing in 3 M KCl solution and it is cleaned with deionized water before it is used.

4.4 Methods

Activation of pencil leads

After leads were brought to certain height, they placed in pencil which was chosen for experiment. The height, remaining out of the pencil is 1.5 cm. This exolead was placed in cell containing Buffer solution and the working electrode was brought ready for analysis. Then the other two electrodes, Platinum as counter electrode and reference electrode which is Ag/AgCl were rinsed with deionized water.

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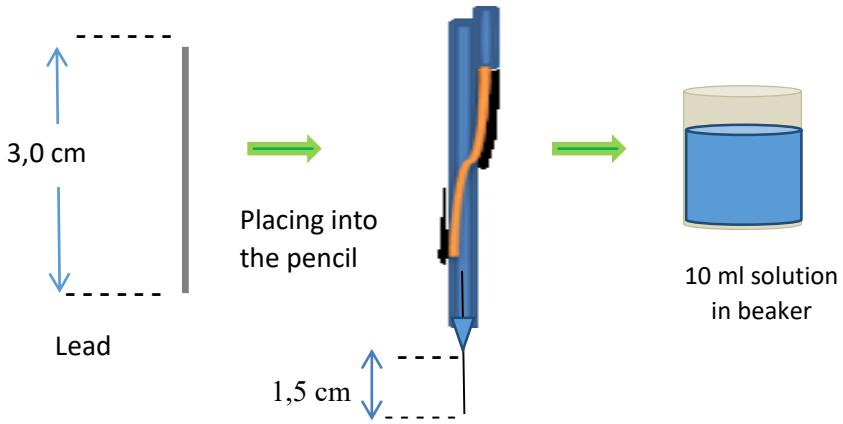


Figure 4.4: View of preparing of working electrode and placing it into the beaker

After placing the lead, the solutions filled to a beaker and other electrodes (reference electrode and counter electrode) with working electrode (PGEl) were placing in the solution. Figure 4.4 shows leads with working electrode. Leads were cut in 3cm height where they are placed in Rotring pen which has conductity

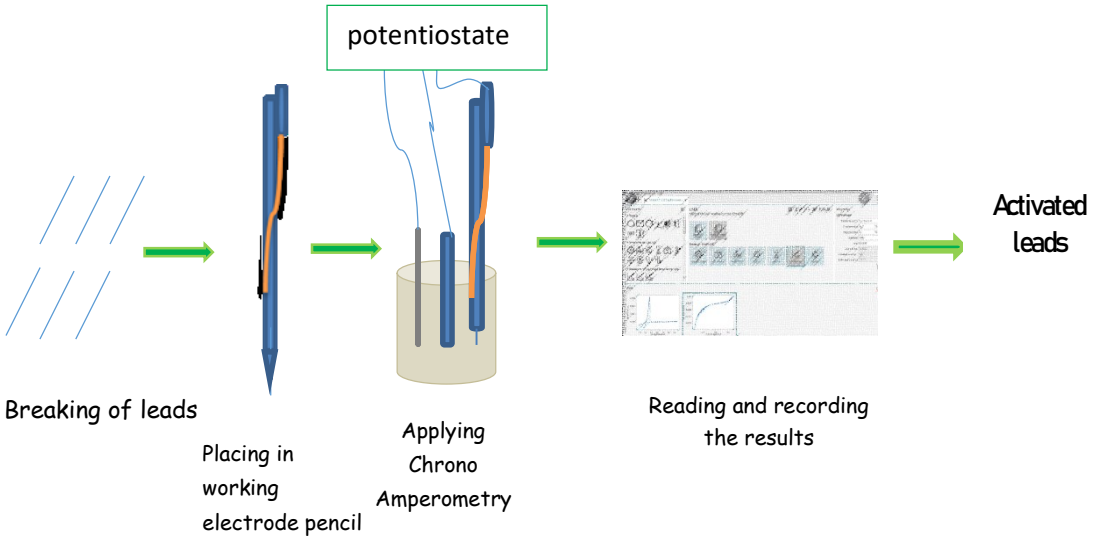


Figure 4.5: Steps of Chrono Amperometry with pencil electrode.

Following that, three electrode were hooked to potentiostate and leads were activated by chrono amperometry. Figure 4.5 shows steps of activation analysis which was applied before DPV or CV.

Holding in Eppendorf tubes

The activated leads were held in Ependorf Tubes. The samples were 200 µl in the tubes. First group analysis was for Evicap capsules which is consisting AT with soy oil, glyserin, at room temperature. Then the analysis was for Evicap capsules which was treated with increasing temperatures starting from room temperature till 150ºC. Second group analysis was for cold pressed olive oil analysis which has a process that has the maximum temperature is about 30- 40ºC and the oil samples were held at room temperature. Then the analysis was for cold pressed olive oil which was treated by increasing temperatures. The third group analysis for cold pressed olive oil which was exposed to sunlight at stable temperature which was held below room temperature .

The samples that were exposured to high temperature were held at room temperature for cooling before analysis. Then every samples are placed in Eppendorf tubes with the amount 200µl and the activated leads were placed in them for 30 minutes and Figure 4.6 shows first step of adsorption of oil on activated leads.

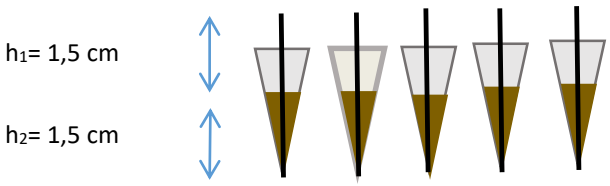


Figure 4.6: Activated leads in Eppendorf Tubes.

Drying of leads

Leads were taken out from Eppendorf tubes and the leads were turned inside out and they were dried for 30 minutes in plastic tube holder.

Applying of cyclic voltammetry

CV was used for setting the interval of the oxidation and the reduction. CV is used for scanning interval of the position of oxidation or reduction of a compound in the sample of solution if it is not found in the literature. If some peaks have seen in graph of CV, it can be passed to the next step. The interval of the next voltammetric method was determined after CV. The upper and lower vertex potential as well as the start and stop potential can be different from upper and lower vertex potential but it can be selected between these interval.

Applying of Differential Pulse Voltammetry

DPV was applied after CV. The start and stop potential were decided in the previous step.

4.5 Temperature Analysis of Cold Pressed Olive Oil

Three types of cold pressed olive oils were analyzed. These are cold pressed olive oils, cold pressed olive oils exposing to sun light and cold pressed olive oils exposing to high temperatures. Additonally one type of olive oil (refined olive oil) were analyzed at room conditions whereas extra virgin olive oils can be acceptable to be same with cold pressed olive oils.

The samples are cold pressed olive oils that are stored at room conditions. And they are seperated to groups. These groups are GR (cold pressed olive olive storing at room temperature), G40 (cold pressed olive oils holding at 40ºC in the oven up to 30min), G50 (cold pressed olive oils holding at 50ºC in the oven up to 30min), Gº60 (cold pressed olive oils holding at 60C in the oven up to 30min), G80 (cold pressed olive oils holding at 80ºC in the oven up to 30min), G100 (cold pressed olive oils holding at 100ºC in the oven up to

30min), G120 (cold pressed olive oils holding at 120ºC in the oven up to 30min), G150 (cold pressed olive oils holding at 150ºC in the oven up to 30min) and G200 (cold pressed olive oils holding at 200ºC in the oven up to 30min).

4.6 Temperature Analysis of Evicap (Vitamin E supplement)

The samples are Evicap that are stored at room conditions. And they were seperated to groups. These groups are GR (Evicap storing at room temperature), G40 (Evicap holding at 40ºC in the oven up to 30min), G50 (Evicap holding at 50ºC in the oven up to 30min), G60 (Evicap holding at 60ºC in the oven up to 30min), G80 (Evicap holding at 80ºC in the oven up to 30min), G100 (Evicap holding at 100ºC in the oven up to 30min), G120 (Evicap holding at 120ºC in the oven up to 30min), G150 (Evicap holding at 150ºC in the oven up to 30min) and G200 (Evicap holding at 200ºC in the oven up to 30min).

4.7 Sunlight Analysis

Cold pressed olive oils are the samples of this analysis. It was desired to catch only sunlight effect in ice-water bath whose temperature is held approximately at 0ºC. Ice water bath contained broken ice cubes and salt (NaCl). Figure 4.7 shows picture of cold pressed olive oil in ice water bath under sunlight for 1 hour.

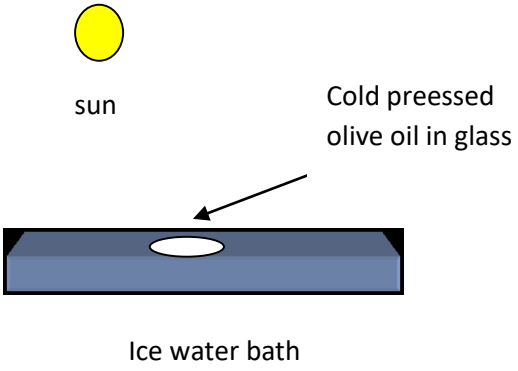


Figure 4.7: Schematic illustration of sunlight analysis

CHAPTER 5

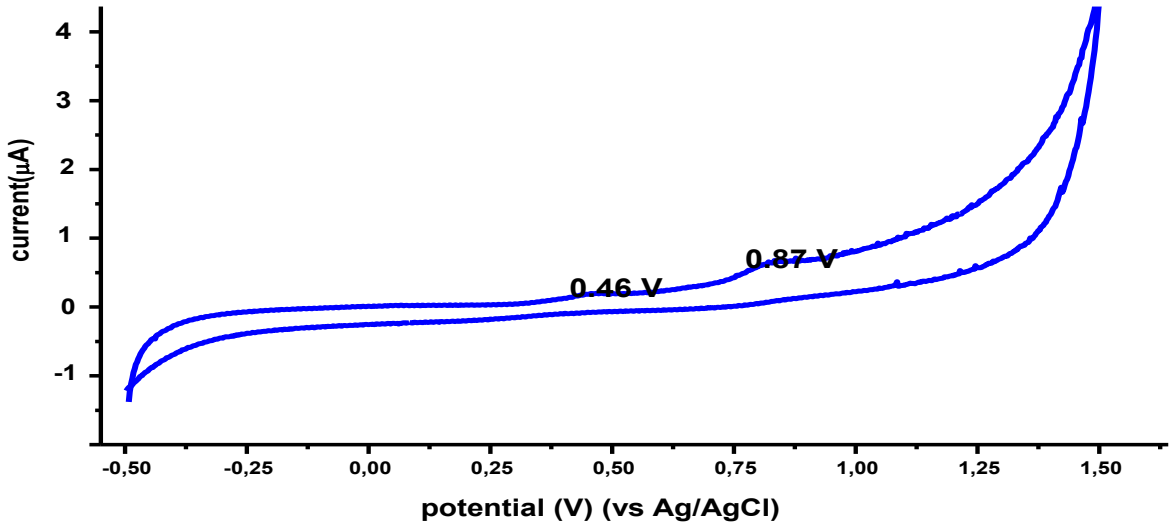
RESULTS AND DISCUSSION

5.1 Cyclic Voltammetry Analysis

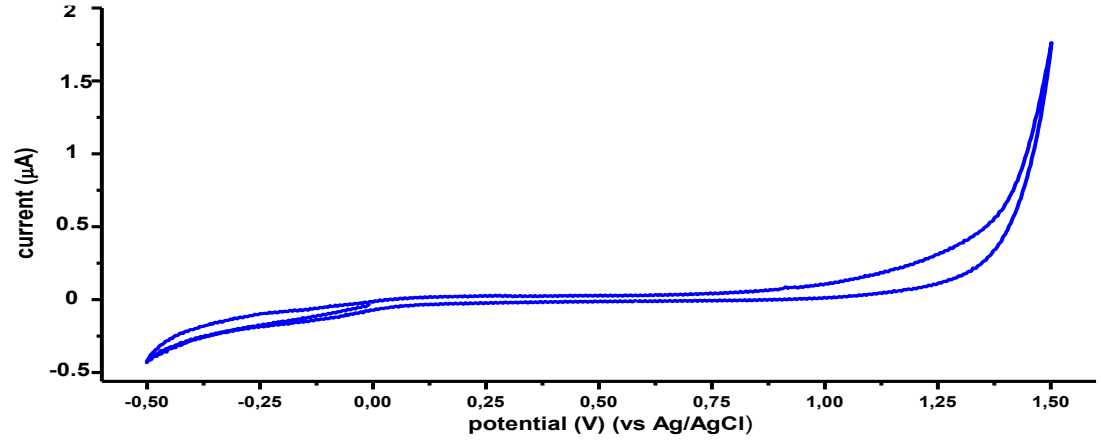
Samples were chosen for CV analysis to detect oxidation and reduction potential of AT. ROO and CPO were analyzed by CV with tripple electrode system, PGEL as working electrode, Ag/AgCl as reference electrode and Platinum as counter electrode. CV procedure was applied with scan rate 0.1 V/s.

α-Tocopherols (ATs) in CPO were adsorbed on PGEL and Figure 5.1(a) shows cyclic voltammogram of CPO. CPO were analyzed by potentiostat with PGEL as working electrode in three electrode system after the sample was adsorbed on PGEL. The potential of anodic peaks were observed in the range of 0.36-0.58 V and 0.77-0.95 V for CPO and there was no peak for ROO analysis. Peak potential of AT was at 0.46 V with peak height of 2.477×10^{-7}A and peak around 0.77-0.95V can be consired as oxidation of phenolic compounds, carotenoids and tocopherols (Tsopelas et al., 2018).

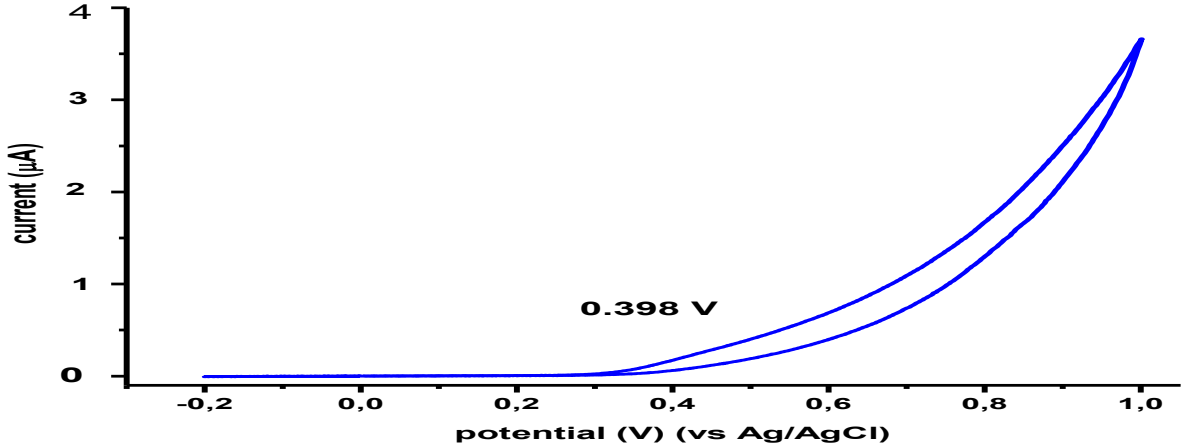
According to the study of Tasan and Demirci, refining process evoked to α, β, γ and δ tocopherol loss of sunflower oils with 30.2, 27.5, 67.3, 88.1 % respectively (Tasan and Demirci, 2004). Results shown in Figure 5 supports that refining process evoked to α, β, γ and δ tocopherol loss.


current(μA)
potential (V) (vs Ag/AgCl)
0.46 V
0.87 V

(a)


current (μA)
potential (V) (vs Ag/AgCl)

(b)


current (μA)
potential (V) (vs Ag/AgCl)
0.398 V

(c)

Figure 5.1. Cyclic Voltammogram of α- tocopherol in **(a)** CPO with PGEL in aqueous ACB solution with 0.1 V/s scan rate; **(b)** ROO with scan rate 0.1 V/s in ACB with PGE as working electrode.**(c)** vitamin E supplement (Evicap 200 IU) with scan rate 0.05 V/s

Figure 5.1. (b) shows voltammogram of ROO. ROO was measured by CV with PGEL as working electrode, Ag/AgCl as reference electrode and Platinum as counter electrode. There was no oxidation peaks of tocopherols in ROO in their voltammograms. It is due to different process of oils for CPO and ROO during their production.

According to Gunstone's book, refined oils can be classified as physical, chemical, cold chemical and modified chemical where this classifications have differentatives for temperature treatments. The temperatures are 120-130ºC at 50 mm Hg in bleaching step in physical refining, 235-245ºC at 3-6 mmHg in deodorization step in physical refining, 150ºC after deodorization in physical refining and is cooled to under 35ºC (Gunstone, 2011). In chemical refining process, the temperature is reached to 85-90ºC before centrifusion for water seperation and 80-90º C is for cold chemical refining process (Gunstone, 2011). In addition to this, crude oil was treated with 55ºC in cold chemical refining process. For the modified chemical refined oils, the oil is heated to 120-130ºC for 30-45 minutes (Gunstone, 2011). To summarize, refining process needs high temperatures compared to ExVO or CPO and high temperature treatment evokes to loss of tocopherols so peak of AT could not been seen in the voltammogram of ROO.

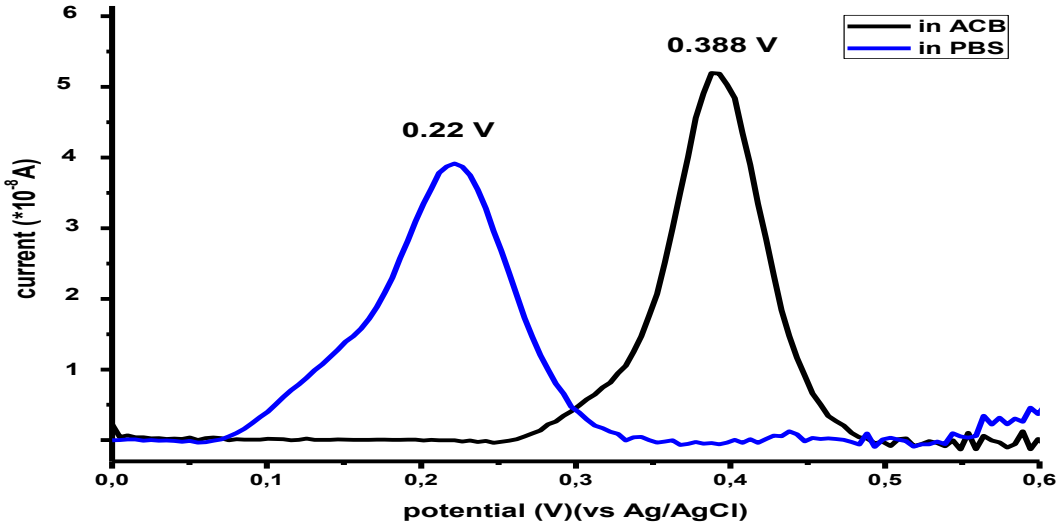
5.2 Differential Pulse Voltammetry Analysis

Samples were chosen and DPV applied with triple electrode system. CPO was detected in PBS and ACB, ViE supplement was detected in ACB and γ-tocopherol was detected in ACB. Figure 5.2 shows diffrential pulse voltammograms of CPO in ACB and PBS, ViE supplement containing AT in ACB and γ-tocopherol in ACB.

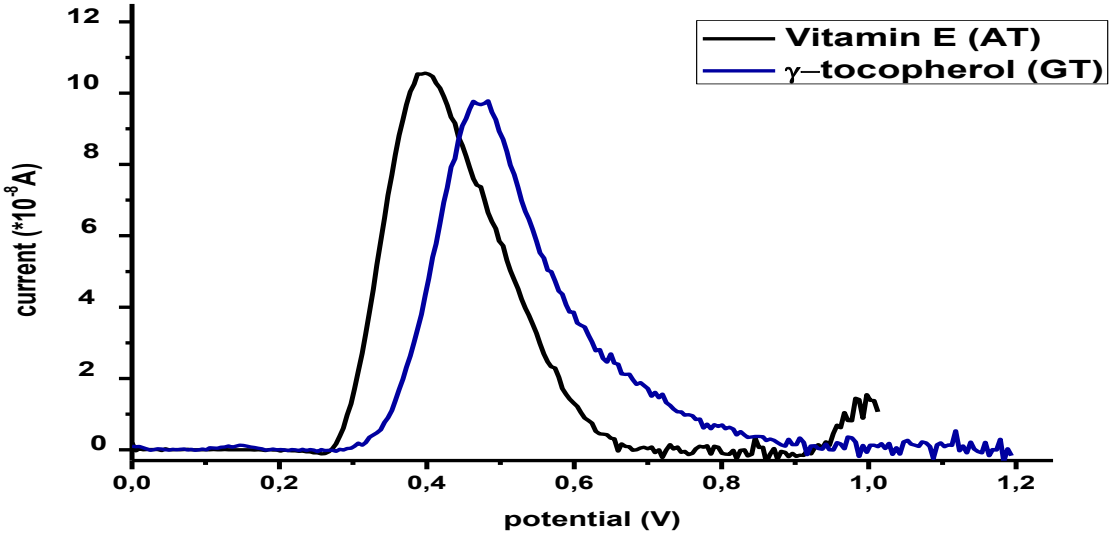
As seen in the Figure 5.2 (a) AT peak of CPO was obtained at the potential of 0.388 V with a peak height of 5.24 x 10^{-8} A in ACB. Whereas oxidation peak of AT was obtained at the potential of 0.2 V with a peak height of 3.9 x 10^{-8} in PBS (phosphate buffer solution). Peak

position was differed with pH of solution and it was shifted to left in basic solution in voltammogram.

In a research conducted by Webster AT was oxidized at about 0.5 V (± 1 V) vs. ferrocene in non-acidic or non-basic environment (Webster, 2011) whereas AT in CPO was analyzed in ACB (pH 4.8) and PBS (pH 7.4) vs. Ag/AgCl . The peaks were observed at around 0.388 V in ACB and 0.22 V in PBS so pH affected the position of oxidation peak.



(a)



(b)

Figure 5.2. Differential Pulse Voltammograms of **(a)** CPO in ACB (pH 4.8) and in PBS (pH 8) **(b)** ViE supplement containing α-tocopherol and γ-tocopherol standard in ACB.

ViE (134mg in a capsule) supplement including AT was analyzed and Figure 5.2(b) shows a peak around 0.4 V on the graph where has peak height was observed $11x10^{-8}$A. There appears to be oxidation in the observed area.

γ- Tokoferol (GT) was diluted with refined olive oil and Figure 5.2(b) shows the oxidation peak around 0.48 V with peak height (current) 9.55 $x10^{-6}$A.

5.3 Standard of α-Tocopherol Analysis

A-tocopherol standard was supplied from Sigma Aldrich and diluted in cold pressed olive oil brought from local fabricate. DPV was applied to detect peak heights of AT in CPO and Figure 5.3 shows peak heights of AT according to concentration of AT.

The standard of AT was added in CPO with concentration of 500, 250, 125, 62.5 and 31.25 ppm. Oxidation peaks of AT was established at potential of 0.4 V. Peak heights was measured according to concentration of AT in CPO. 500 ppm of AT has the highest avarage peak height of 3.2 $x10^{-7}$A and the lowest avarage peak height of 4.5 $x10^{-8}$ was found in 62.5 ppm.

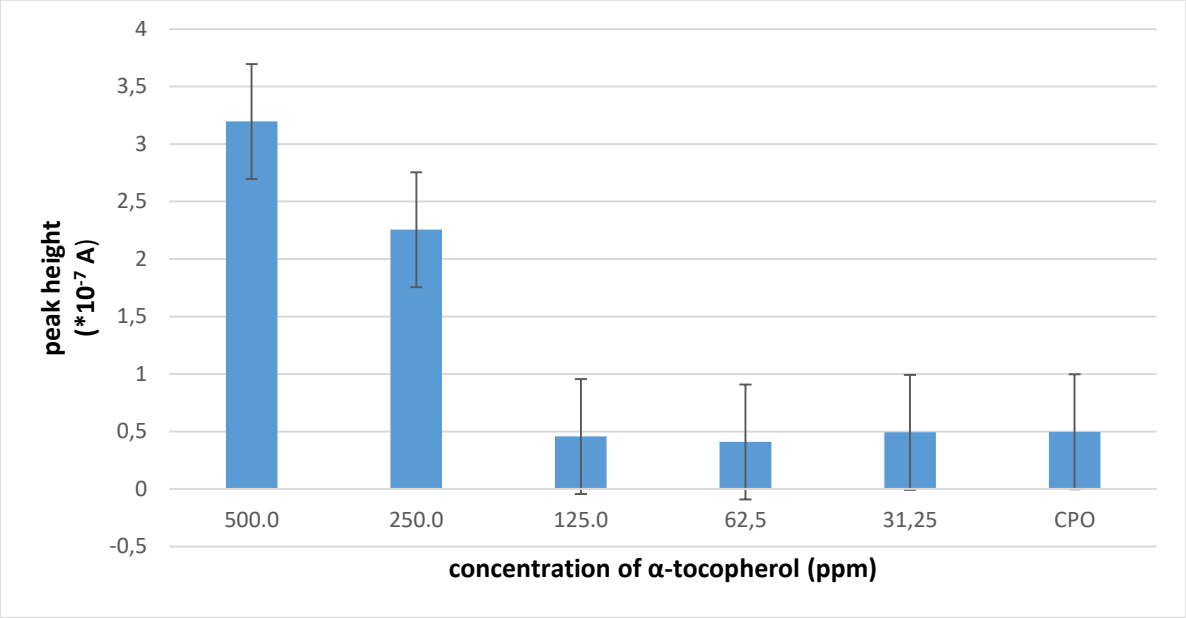


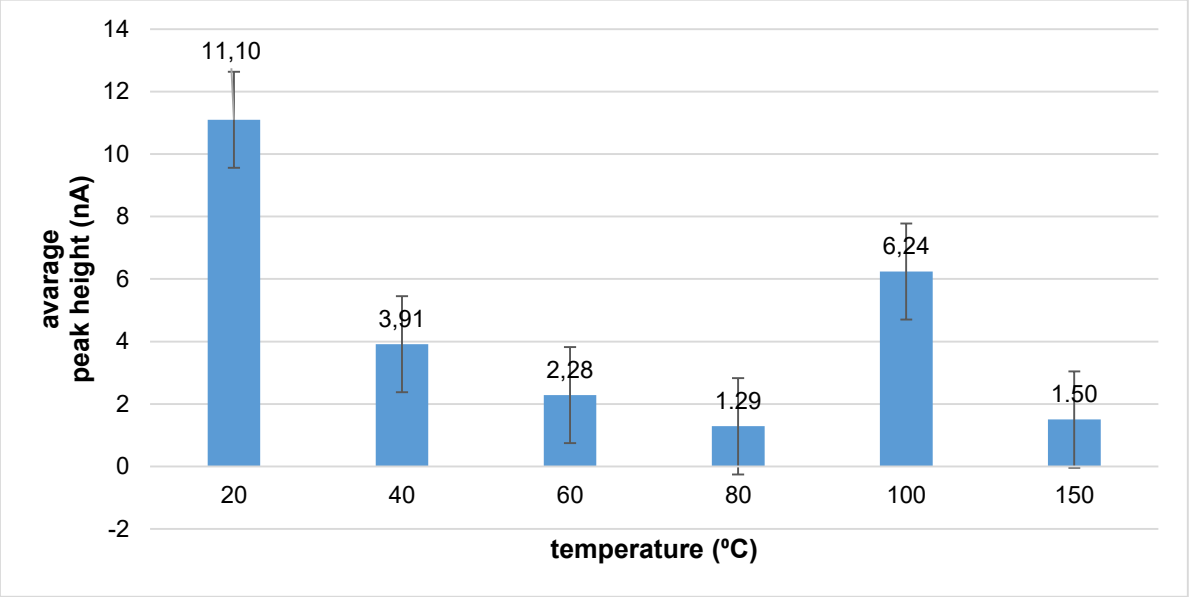
Figure 5.3: Histogram of peak height at 0.4 V (±0.01) according to concentration of AT.

5.4 Temperature Analysis

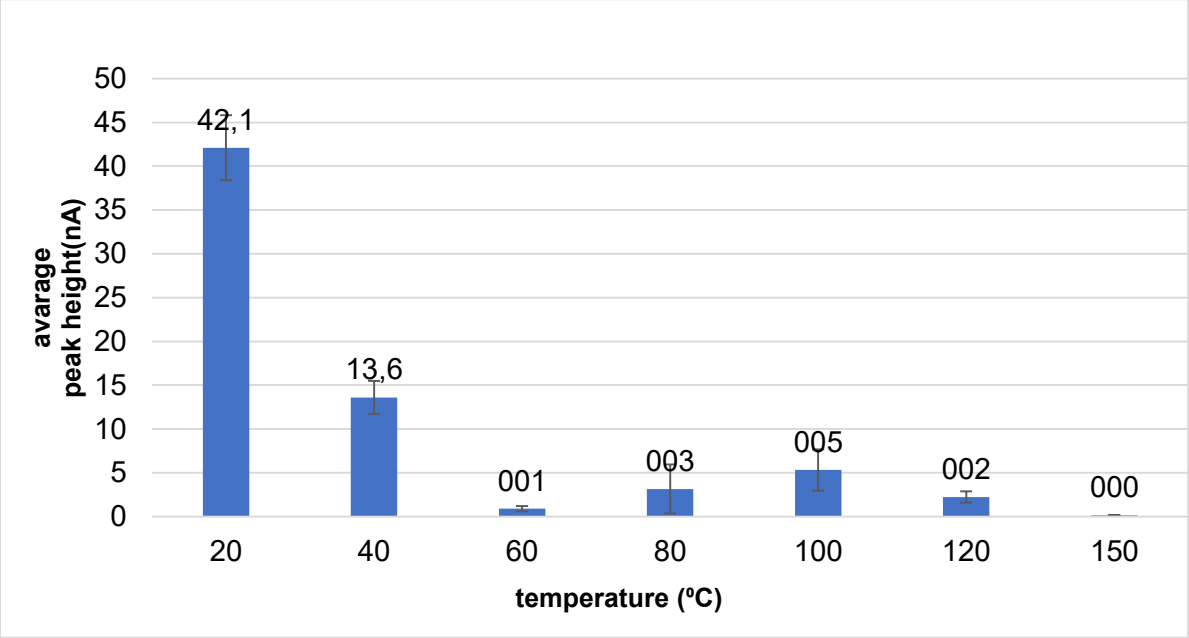
AT is resistant to high temperature in the absence of oxygen however it can be oxidized and degraded in the atmosphere (Sabliov et al., 2009). The samples was heated and held to reach to room temperature and then they analyzed by DPV after pencil graphite was prepared.

Figure 5.4 (a) shows avarage peak heights of AT in nA according to increasing temperatures 20º C, 40º C, 60º C, 80º C, 100º C and 150º C. Vitamin E supplement (AT) was heated to 40º C, 60º C, 80º C, 100º C and 150º C for approximately 30 minutes in the oven and the samples were kept for few minutes outside of the oven to reach to room temperature. Then activated lead was immersed in the samples for 30 minutes and analyzed by DPV.

11.2 nA for 20º C, 3.91 nA for 40º C, 2.28 nA for 60º C, 1.29 nA for 80º C, 6.24 nA for 100ºC and 1.50 nA for 150ºC were measured by DPV with triple electrode system whereas two highest average peak height were at 20º C and 100º C. AT could not be degraded at 20º C but started to degrade from 40º C to 80º C gradually and the highest degradation was observed at 150º C.



(a)



*There is no peak around 0.4V for 200C analysis

(b)

Figure 5.4. Histogram of peak height of **(a)** Vitamin E supplement (Evicap) according to increasing temperature **(b)** CPO according to increasing temperature

Figure 5.4 (b) shows peak heights of AT in CPO. The highest two peaks were at 20º C and 40º C with the peak height of 42.1 and 13.6 nA respectively. The two lowest peak height were at 60º C and 150º C with 0.14 nA and 0.91 nA. The peak heights were increased slowly between 60º and 100º C and started to decreased until the lowest peak height.

5.5 Sun-light effect on Cold Pressed Olive Oil

Cold pressed olive oil which was kept at room temperature and under sunlight was analyzed. AT which was found free in cold pressed olive oil oxidized in range 0.3 and 0.5. CPO which was exposured to sunlight in ice-water bath for 1 hour in the midday was analyzed. DPV was applied with start-stop potential 0.1-0.9V and scan rate 0.010071 V/s. These two signals were overlapped in the Figure 5.5.

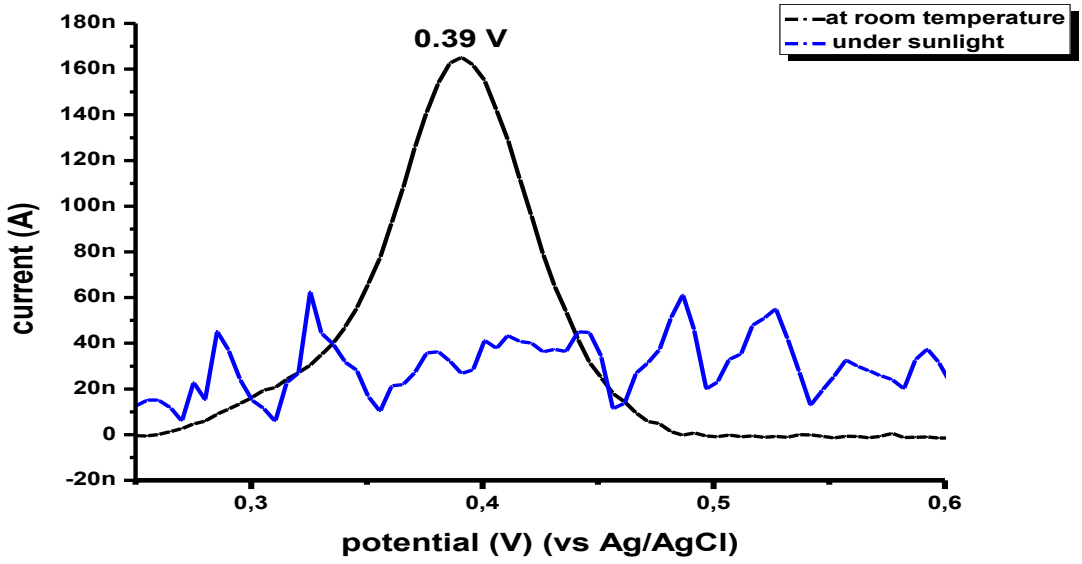


Figure 5.5. DPV analysis of CPO with tocopherol peak at room temperature and keeping under sun light for 1 hour.

The graph shows peak positions of samples of CPO which was stored at room temperature and which was exposured to sunlight. The peak of CPO which was exposured to sunlight was lost at 0.387 V where AT can be oxidized. It was the proof of absence of AT for CPO which was exposured to sunlight for 1 hour in ice water bath.

CHAPTER 6
CONCLUSION

Voltammetric method with three electrode system including pencil graphite electrode as working electrode was applied in order to check temperature and sunlight effect of AT in olive oil. A new method was used for this purpose which involves adsorption of AT on pencil graphite electrodes and consequent voltammetric analysis. This method is quick, sensitive enough and cost-effective in order to check AT levels and environmental effects of AT in CPO.

AT of CPO could be adsorbed on pencil graphite electrode and was detected by three electrode system including a pencil graphite electrode as working electrode. Furthermore AT can be detected in CPO without any extraction prosedure by pencil electrode system. Addition to this, sun-light and temperature effect on cold pressed olive oil can be specified by voltammetry through this method.

The oxidation were observed around 0.4V and 0.87V as potential for cold pressed olive oil. The first oxidation can be indicated as AT oxidation and the second can be concerned as phenolic oxidation (Rodrı´guez-Me´ndez et al., 2008; Diaz et al., 2004).

The another results of our study showed that the amount of AT in cold pressed olive oil CPO decreases with increasing temperature where a slight increase observed at 100 °C which may indicate a compositional change in olive oil at 100 °C. Addition to this, vitamin E supplement containing AT was heated to 40, 60, 80 °C and observed decrease in peak heights which shows oxidation of AT at 0.4V whereas there is an increase in peak heights at 0.4V as a result of AT oxidation and oxidation product was formed at 100°C. 10 parallel analysis were done for 100°C and the results were not appeared as accidentally. After that, there was an decrease when the temperature approached to 150°C. As concluded from sun-light experiments AT oxidation signal disappeard after CPO was exposed to sunlight for 1 hour. All these results show that voltammetric techniques can be used successfully by adsorbing AT on graphite and followed by the analysis.

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APPENDIX I:

Fevziye Dindar Thesis

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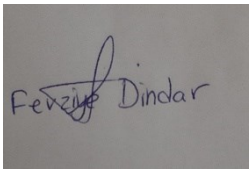

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APPENDIX II: ETHICAL APPROVAL DOCUMENT

NEAR EAST UNIVERSITY

ETHICAL APPROVAL DOCUMENT

Date: 21/1/2021

To the Graduate School of Applied Sciences

For the thesis project entitled as "Investigating Temperature and Sunlight Effects On Cold Pressed Olive Oil Using Voltammetric Determination of A-Tocopherol Level" the researchers declare that they did not collect any data from human/animal or any other subjects. Therefore, this project does not need to go through the ethics committee evaluation.

Title: Assist. Prof. Dr.

Name Surname: Süleyman Aşır

Signature: 

Role in the Research Project: Supervisor